CLAIMS

1. A method of spectrographic measurement, comprising the steps of:

(a) generating light energy using an excitation source, said light energy being caused to fall on a sample to be assayed, causing said sample to output an output optical signal;

(b) generating a plurality of modulation frequencies;

(c) generating a plurality of heterodyne frequencies to form a set of heterodyne signals at said heterodyne frequencies, each of said heterodyne frequencies being associated with one of said modulation frequencies;

(d) coupling said modulation frequencies to said excitation source, causing said excitation source to generate excitation energy modulated in intensity in proportion to said modulation frequencies;

(e) sampling a portion of said laser excitation energy to form a reference laser excitation signal;

(f) focusing said output optical signal as an image modulated with said plurality of modulation frequencies on an image intensifier;

(g) intensifying said image to form an intensified image modulated with said plurality of modulation frequencies;

(h) receiving said intensified image modulated with said plurality of modulation frequencies on a multielement optical detector;

(i) generating a plurality of measurement signals using said multielement optical detector, each measurement signal associated with a single one of said elements;

(j) for each measurement signal associated with a single one of said elements of said multielement optical detector, mixing said measurement signal with said heterodyne signal to generate a plurality of low-frequency measurement modulation products, one low-frequency measurement modulation product being associated with each of said modulation frequencies and comprising

the difference between a single modulation frequency and its associated heterodyne frequency and having a measurement amplitude and phase;

(k) mixing said reference laser excitation energy with said heterodyne signal to generate a plurality of reference modulation products, one reference modulation product being associated with each of said modulation frequencies and comprising the difference between a single modulation frequency and its associated heterodyne frequency and having a reference amplitude and phase, each of said low-frequency reference modulation products being associated with one of said measurement modulation products;

(l) for each of said plurality of low-frequency measurement modulation products, comparing said low-frequency measurement modulation product to its associated low-frequency reference modulation product to generate an output signal indicating characteristics of said sample at the region on said sample associated with each of said elements.

2. The method of claim 1, wherein said output signal is numerically processed to generate changes over time.

3. The method of claim 1, wherein said output signal may be graphically displayed.

4. The method of claim 1, wherein said output signal is numerically processed to generate a desired parameter.

5. The method of claim 1, wherein said excitation source is a laser.

6. The method of claim 1, wherein said output optical signal comprises fluorescent energy from said sample

7. The method as in claim 1, wherein said modulation frequencies are harmonically related.

8. The method as in claim 1, wherein excitation source is a laser whose output is modulated by a Pockel's cell.

9. The method as in claim 1, wherein excitation source is a laser whose output is a pulsed laser.

10. The method as in claim 9, wherein said laser is a pulsed-dye laser.

11. The method as in claim 1, wherein excitation source is a light emitting diode.

12. The method as in claim 1, wherein reference modulation products are the low-frequency reference modulation products output during said mixing operation.

13. The method as in claim 1, wherein said comparison is done by measuring the relative phase and amplitude of said low-frequency measurement modulation product as compared to said low-frequency reference modulation product and generating a modulation data point and a phase data point;

14. The method as in claim 13, further comprising:

(m) for each element, fitting said modulation data points to a first curve using the method of least squares;

(n) for each element fitting said phase data points to a second curve using the method of least squares;

(o) comparing said first and second curves to a database to determine characteristics of said sample; and

(p) displaying said characteristics.

15. The method of claim 1, wherein before said excitation energy output by said excitation source is caused to fall on said sample to be measured, the system is calibrated by first using, in place of said sample, a standard consisting of a zero lifetime scattering solution to create a set of normalizing phase and modulation standard values against which said phase and modulation values for said sample our measured.

16. A method of spectrographic measurement, comprising the steps of:

(a) generating light energy using an excitation source, said light energy being caused to fall on a sample to be assayed, causing said sample to output an output optical signal;

(b) generating a plurality of modulation frequencies;

(c) generating a plurality of heterodyne frequencies to form a set of heterodyne signals at said heterodyne frequencies, each of said heterodyne frequencies being associated with one of said modulation frequencies;

(d) coupling said modulation frequencies to said excitation source, causing said excitation source to generate excitation energy modulated in intensity in proportion to said modulation frequencies;

(e) sampling a portion of said laser excitation energy to form a reference laser excitation signal;

(f) focusing said output optical signal as an image modulated with said plurality of modulation frequencies on an image intensifier;

(g) intensifying said image to form an intensified image modulated with said plurality of modulation frequencies;

(h) receiving said intensified image modulated with said plurality of modulation frequencies on a multielement optical detector;

(i) generating a plurality of measurement signals using said multielement optical detector, each measurement signal associated with a single one of said elements;

(j) for each measurement signal associated with a single one of said elements of said multielement optical detector, comparing the output of said elements to a standard to generate an output signal indicating characteristics of said sample at the region on said sample associated with each of said elements.

17. Apparatus for performing fluorescence measurement, comprising:

(a) a light source generating laser excitation energy, oriented to illuminate a sample to be measured and cause said sample to emit fluorescent energy;

(b) a frequency generator generating a plurality of modulation frequencies and a plurality of heterodyne frequencies, each of said heterodyne frequencies being associated with one of said modulation frequencies said frequency generator being coupled to said excitation source, whereby said source generates excitation energy modulated in intensity in proportion to said modulation frequencies;

(c) and optical member positioned to receive said laser excitation energy and divert a portion of said laser excitation energy, said portion of said laser excitation energy forming a reference laser excitation signal;

(d) focusing optics positioned to receive said fluorescent energy and form an image modulated with said plurality of modulation frequencies;

(e) an image intensifier positioned to receive said image, said image intensifier having an output for outputting an intensified image modulated with said plurality of modulation frequencies;

(f) a multielement optical detector positioned to receive said intensified image modulated with said plurality of modulation frequencies and generating response thereto a plurality of measurement signals, each associated with a single one of said elements;

(g) a mixer coupled to receive each of said measurement signals and each of said heterodyne signals and producing in response to said measurement signals and said heterodyne signals a plurality of low-frequency

measurement modulation products, one low-frequency measurement modulation product being associated with each of said modulation frequencies and comprising the difference between a single modulation frequency and its associated heterodyne frequency and having a measurement amplitude and phase; and

(h) a mixer coupled to said reference laser excitation signals and said heterodyne signals to generate a plurality of low-frequency reference modulation products, one low-frequency reference modulation product being associated with each of said modulation frequencies and comprising the difference between a single modulation frequency and its associated heterodyne frequency and having a reference amplitude and phase, each of said low-frequency reference modulation products being associated with one of said measurement modulation products, each of said low-frequency measurement modulation products, and their associated low-frequency reference modulation products indicating phase and the modulation information.

18. Apparatus as in claim 17, wherein optical member is a partially silvered mirror.

19. Apparatus as in claim 17, wherein optical member is a prism.

20. Apparatus for performing fluorescence measurements, comprising:

(a) a light source generating laser excitation energy, oriented to illuminate a sample to be measured and cause said sample to emit fluorescent energy;

(b) a frequency generator generating a plurality of modulation frequencies and a plurality of heterodyne frequencies, each of said heterodyne frequencies being associated with one of said modulation frequencies said frequency generator being coupled to said excitation source, whereby said source generates excitation energy modulated in intensity in proportion to

said modulation frequencies;

(c) and optical member positioned to receive said laser excitation energy and divert a portion of said laser excitation energy, said portion of said laser excitation energy forming a reference laser excitation signal;

(d) focusing optics positioned to receive said fluorescent energy and form an image modulated with said plurality of modulation frequencies;

(e) an image intensifier positioned to receive said image, said image intensifier having an output for outputting an intensified image modulated with said plurality of modulation frequencies;

(f) a multielement optical detector positioned to receive said intensified image modulated with said plurality of modulation frequencies and generating response thereto a plurality of measurement signals, each associated with a single one of said elements; and

(g) a calculating device coupled to said measurement signals, said heterodyne signals and said reference laser excitation signals and configured to extract phase and the modulation information.

21. Apparatus as in claim 20, wherein said calculating device is a computer.

22. Apparatus as in claim 20, wherein said focusing topics are microscope optics.

23. Apparatus as in claim 22, wherein said microscope optics are confocal optics. analyzed.

24. A method of fluorescence measurement, comprising the steps of:

(a) generating light energy in the form of laser excitation energy output by an excitation source, said laser excitation energy being caused to fall on a sample to be measured and cause said sample to emit fluorescent energy;

(b) generating a plurality of modulation frequencies;

(c) generating a plurality of heterodyne frequencies to form a set of heterodyne signals at said heterodyne frequencies, each of said heterodyne frequencies being associated with one of said modulation frequencies;

(d) coupling said modulation frequencies to said excitation source causing said source to generate excitation energy modulated in intensity in proportion to said modulation frequencies;

(e) sampling a portion of said laser excitation energy to form a reference laser excitation signal;

(f) focusing said fluorescent energy as an image modulated with said plurality of modulation frequencies on an image intensifier;

(g) intensifying said image to form an intensified image modulated with said plurality of modulation frequencies;

(h) receiving said intensified image modulated with said plurality of modulation frequencies on a multielement optical detector;

(i) generating a plurality of measurement signals using said multielement optical detector, a single signal being output from each of the elements of said multielement optical detector, each measurement signal associated with a single one of said elements;

(j) for each measurement signal associated with a single one of said elements of said multielement optical detector, mixing said measurement signal with said heterodyne signal to generate a plurality of low-frequency measurement modulation products, one low-frequency measurement modulation product being associated with each of said modulation frequencies and comprising the difference between a single modulation frequency and its associated heterodyne frequency and having a measurement amplitude and phase;

(k) mixing said reference laser excitation signal with said heterodyne signal to generate a plurality of low-frequency reference modulation products, one low-frequency reference modulation product being associated with each of said modulation frequencies and comprising the difference between a single

modulation frequency and its associated heterodyne frequency and having a reference amplitude and phase, each of said low-frequency reference modulation products being associated with one of said measurement modulation products;

(l) for each of said plurality of low-frequency measurement modulation products, comparing said low-frequency measurement modulation product to its associated low-frequency reference modulation product to measure the relative phase and amplitude of said low-frequency measurement modulation product as compared to said low-frequency reference modulation product and generating a modulation data point and a phase data point;

(m) for each element, fitting said modulation data points to a first curve using the method of least squares;

(n) for each element fitting said phase data points to a second curve using the method of least squares;

(o) comparing said first and second curves to a database to determine characteristics of said sample; and

(p) displaying said characteristics.

25. The method of claim 24, wherein before said excitation energy output by said excitation source is caused to fall on said sample to be measured, the system is calibrated by first using, in place of said sample, a standard consisting of a zero lifetime scattering solution to create a set of normalizing phase and modulation standard values against which said phase and modulation values for said sample our measured.

26. The method of claim 25, wherein said normalizing phase and modulation standard values are generated by the steps of:

(q) causing said generated light energy in the form of laser excitation energy output by said excitation source, said laser excitation energy being caused to

fall on a zero lifetime standard, causing said sample to output a reference standard optical signal;

(r) generating a plurality of modulation frequencies;

(s) generating a plurality of heterodyne frequencies to form a a set of heterodyne signals at said heterodyne frequencies, each of said heterodyne frequencies being associated with one of said modulation frequencies;

(t) coupling said modulation frequencies to said excitation source causing said source to generate excitation energy modulated in intensity in proportion to said modulation frequencies;

(u) sampling a portion of said laser excitation energy to form a reference laser excitation signal;

(v) focusing said reference standard optical signal as a standard image modulated with said plurality of modulation frequencies on said image intensifier;

(w) intensifying said standard image to form an intensified standard image modulated with said plurality of modulation frequencies;

(x) receiving said intensified standard image modulated with said plurality of modulation frequencies on said multielement optical detector;

(y) generating a plurality of measurement signals using said multielement optical detector, a single signal being output from each of the elements of said multielement optical detector, each measurement signal associated with a single one of said elements;

(z) for each measurement signal associated with a single one of said elements of said multielement optical detector, mixing said measurement signal with said heterodyne signal to generate a plurality of low-frequency measurement modulation products, one low-frequency measurement modulation product being associated with each of said modulation frequencies and comprising the difference between a single modulation frequency and its associated heterodyne frequency and having a measurement amplitude and phase;

(aa) mixing said reference laser excitation signal with said heterodyne signal to generate a plurality of low-frequency reference modulation products, one low-frequency reference modulation product being associated with each of said modulation frequencies and comprising the difference between a single modulation frequency and its associated heterodyne frequency and having a reference amplitude and phase, each of said low-frequency reference modulation products being associated with one of said measurement modulation products;

(bb) for each of said plurality of low-frequency measurement modulation products, comparing said low-frequency measurement modulation product to its associated low-frequency reference modulation product to measure the relative phase and amplitude of said low-frequency measurement modulation product as compared to said low-frequency reference modulation product and generating a reference standard modulation data point and a reference standard phase data point.